Identification of Capsular Serotypes K1 and K2 in Clinical Isolates of Klebsiella Pneumoniae in North of Iran

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ABSTRACT

Background and Objectives: Klebsiella pneumoniae is one of the most important nosocomial pathogens. Its capsular polysaccharide is considered as the first and most important virulence factor of this bacterium. This study aimed to investigate the presence of capsular serotypes K1 and K2 in K. pneumoniae isolates to examine the virulence potency of the isolates.

Methods: Overall, 65 capsulated K. pneumoniae isolates were collected from patients with urinary tract infections in Rasht, Iran. The isolates were examined using biochemical tests and CPS gene amplification using PCR. Mucoid phenotype of the isolates was determined by the string test. The presence of K1 and K2 genes was evaluated by PCR using specific primers for the genes.

Results: Of 65 K. pneumoniae isolates, seven (10.77%) were positive for the presence of the K1 gene and four (6.15%) were positive for the presence of the K2 gene. In addition, six serotype K1 isolates (27.27%), four serotype K2 isolates (18.18%), and 12 non-K1/K2 serotype isolates (54.54%) had hypermucoviscosity phenotypes.

Conclusion: Our results confirm the presence of the capsular serotypes in K. pneumoniae isolates, with a relatively high prevalence for the capsular serotype K1. This study clarifies the importance of rapid diagnosis and suitable treatment of infections caused by K. pneumoniae in prevention of complicated infections.

Keywords: Klebsiella pneumoniae, Virulence factors, Capsular polysaccharide.

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INTRODUCTION

Klebsiella pneumoniae is an opportunistic and important nosocomial pathogen that causes a wide range of infections including septicaemia, pneumonia, urinary tract infection, meningitis and abscesses in different organs, particularly liver (1). Klebsiella sp. are surrounded by a thick layer of hydrophilic capsular polysaccharide (CPS), which is considered as the first and most important virulence factor of these bacteria. CPS has an essential role in the attachment of bacteria to epithelial and mucosal surfaces. It also protects the bacteria from serum opsonization and phagocytosis, thereby hiding the bacteria from the host immune system. On the other hand, the capsule provides protection against adverse environmental conditions and reduces the permeability of antibiotics into the bacteria (2, 3). Capsular serotypes K1 and K2 strains of K. pneumoniae have more pathogenicity. These serotypes have shown more resistance against the bactericidal effect of serum and phagocytic ingestion compared to other serotypes (4). Therefore, the presence of K_1 and K_2 capsular serotypes of K. pneumoniae in clinical samples may be a sign of serious infections. The present study aimed at investigating the presence of K_1 and K_2 capsular serotypes of K. pneumoniae to examine the virulence potency of the clinical isolates of this bacterium.

MATERIAL AND METHODS

Overall, 65 K. pneumoniae isolates from patients with urinary tract infections were collected from clinical laboratories in city of Rasht, Iran. Bacterial genomic DNA was extracted using commercial DNA isolation kits for gram-negative bacteria (Cinnagen, Iran). All isolates were tested for the presence of K. pneumoniae capsular antigens by PCR, using CPS-specific primers (5). In addition, primers specific for capsular serotype K1- and K2-specifying genes were used in the PCR experiment. All PCR experiments were performed according to the method described by Feizabadi et al. (6). Table 1 shows the primers used in the PCR experiments.

Table 1: Oligonucleotide primers used for amplification of sequences specific for *K. pneumonia* CPS, and K1 and K2 genes.

Gene	Primers	ılicon size (bp)
CPS	ΓATTCATCAGAAGCACGAGCTGGGAGAAGCC-3′	418
	GTCGGTAGCTGTTAAGCCAGGGGCGGTAGCG-3́	
K1	F: 5′ ACGATAGAGGTGTATTGTCGC 3’	352
	R: 5′ACGATAGAG GTGTATTGTCGC3′	
K2	R:5' TGATACTTGACAGAGGGAGTA-5'	321
	F: 5'ACGATCGTTACAGTGACAAG-3'	

RESULTS

In this study, all 65 K. pneumoniae isolates were gram-negative, non-motile, rod-shaped, lactose-fermenting, methyl-red negative, Voges–Proskauer positive, indol negative, catalase positive, urease positive and hydrogen sulphide negative, with mucoid colonies on MacConkey agar. The presence of K. pneumoniae CPS was confirmed by detecting a 418 bp fragment after the PCR experiment (Figure 1). The specific primers yielded a 352 bp fragment in seven isolates (10.77%), which were identified as capsular serotype K1. In addition, a 321 bp fragment was produced in four isolates (6.15%), which were identified as capsular serotype K2. Moreover, 54 isolates (83.07%) were identified as non-K1/K2 serotypes (Figure 2).

Figure 1- Agarose gel electrophoresis of CPS gene PCR amplicons. Column M: 100bp DNA marker, Column 1: Positive control (418 bp amplicon of CPS confirmed by sequencing), Columns 2-6: CPS positive strains.

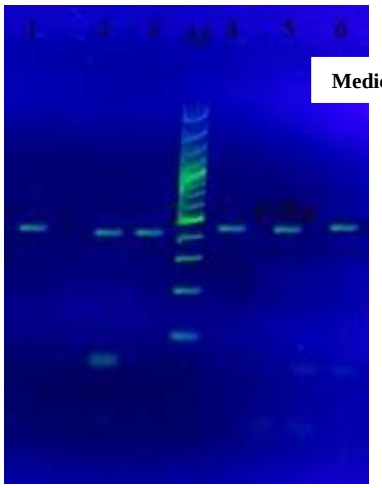

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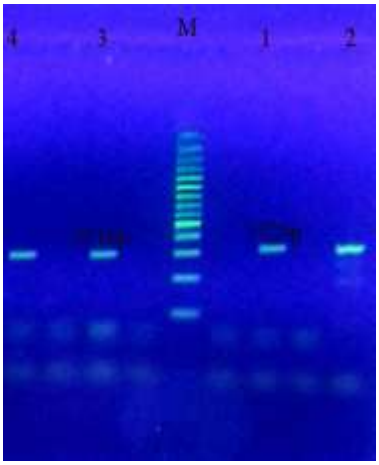
Figure 2- Agarose gel electrophoresis of PCR amplicons for K1- and K2-specifying genes. Column M: 100bp DNA marker, Column 1: Positive control (352 bp amplicon of K1 confirmed by sequencing), Column 2: K1 positive strain, Column 3: Positive control (321 bp amplicon of K2 confirmed by sequencing), Column 4: K2 positive strain.



DISCUSSION

In the present study, 65 K. pneumoniae clinical isolates were examined to identify the K1 and K2 capsular serotypes. The results showed that seven isolates (10.77%) were serotype K1, while four (6.15%) were serotype K2. In a similar study in Iran, Feizabadi et al. (2013) reported the frequency of capsular serotypes K1 and K2 among 89 K. pneumoniae isolates from urine samples as 11.2% and 14.6%, respectively (6). Similar studies have been conducted in other parts of the world. In study of Yeh et al. on 50 K. pneumoniae clinical isolates from patients with liver abscesses in Taiwan, 26 isolates (52%) were identified as serotype K1 and 10 isolates (20%) were identified as serotype K2 (7). The results of these studies also showed that non-K1/K2 serotypes are involved in aggressive Klebsiella infections. Another study in Taiwan by Liao et al. (2011) demonstrated that K1 is the most abundant serotype among Klebsiella isolates from cases of bacteraemia (8). In Iraq, study of Abdol-Razzaq et al. (2014) on 158 Klebsiella isolates found K1 and K2 capsular antigens in 18.6% and 32.5% of isolates, respectively. However, they found three cases that were positive for both serotypes (5). In a study by Tamara et al. on 40 Klebsiella isolates, 57.5% of isolates were identified as serotype K1, 27.5% were identified as serotype K2, and 15% were identified as non-K1/K2 serotypes (9). Study of Jiang Wang et al. on 101 Klebsiella isolates reported the prevalence of capsular serotypes K1, K2 and non-K1/K2 as 42.57%, 36.63% and 20.79%, respectively (10).

CONCLUSION

Capsular serotype K1 or K2 plays important roles in the pathogenesis of K. pneumoniae infections. Our study confirms the presence of these capsular serotypes in isolates from urine samples, with a relatively high prevalence for the serotype K1. This study clarifies the importance of rapid diagnosis and suitable treatment of infections caused by K. pneumoniae in prevention of complicated infections.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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